PATENT COOPERATION TREATY

PCT

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INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY
(Chapter II of the Patent Cooperation Treaty)

07 MAR 2005

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION See Form PCT/IPEA/416	
UMG-0004.PCT		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/SE2003/001381	04.09.2003	04.09.2002
International Patent Classification (IPC) or national classification and IPC		
C12Q 1/18, C12N 15/74, C07C 235/64, C07C 243/38, A61K 31/167, 31/15// (C12Q 1/18, C12R 1:01), (C12N 15/74, C12R 1:01)		
Applicant		
Innate Pharmaceuticals AB et al		

1. This report is the international preliminary examination report, established by this International Preliminary Examining Authority under Article 35 and transmitted to the applicant according to Article 36.
2. This REPORT consists of a total of 9 sheets, including this cover sheet.
3. This report is also accompanied by ANNEXES, comprising:
   a. ☒ *(sent to the applicant and to the International Bureau)* a total of 9 sheets, as follows:
      ☒ sheets of the description, claims and/or drawings which have been amended and are the basis of this report and/or sheets containing rectifications authorized by this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions).
      ☐ sheets which supersede earlier sheets, but which this Authority considers contain an amendment that goes beyond the disclosure in the international application as filed, as indicated in item 4 of Box No. I and the Supplemental Box.
   b. ☐ *(sent to the International Bureau only)* a total of (indicate type and number of electronic carrier(s)) _____, containing a sequence listing and/or tables related thereto, in computer readable form only, as indicated in the Supplemental Box Relating to Sequence Listing (see Section 802 of the Administrative Instructions).
4. This report contains indications relating to the following items:
   - ☒ Box No. I Basis of the report
   - ☐ Box No. II Priority
   - ☒ Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - ☐ Box No. IV Lack of unity of invention
   - ☒ Box No. V Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - ☐ Box No. VI Certain documents cited
   - ☐ Box No. VII Certain defects in the international application
   - ☒ Box No. VIII Certain observations on the international application

Date of submission of the demand	Date of completion of this report
05.04.2004	23.12.2004
Name and mailing address of the IPEA/SE Patent- och registreringsverket Box 5055 S-102 42 STOCKHOLM Facsimile No. +46 8 667 72 88	Authorized officer Per Renström/EÖ Telephone No: +46 8 782 25 00

Form PCT/IPEA/409 (cover sheet) (January 2004)

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2003/001381

Box No. I Basis of the report

1. With regard to the language, this report is based on the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ This report is based on a translation from the original language into the following language _____, which is the language of a translation furnished for the purposes of:

☐ international search (under Rules 12.3 and 23.1(b))

☐ publication of the international application (under Rule 12.4)

☐ international preliminary examination (under Rules 55.2 and/or 55.3)

2. With regard to the elements of the international application, this report is based on *(replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report):*

☐ the international application as originally filed/furnished

☒ the description:

pages 1-22 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☒ the claims:

pages _____ as originally filed/furnished

pages* _____ as amended (together with any statement) under Article 19

pages* 1-9 received by this Authority on 02-09-2004

pages* _____ received by this Authority on _____

☒ the drawings:

pages 1-12 as originally filed/furnished

pages* _____ received by this Authority on _____

pages* _____ received by this Authority on _____

☐ a sequence listing and/or any related table(s) – see Supplemental Box Relating to Sequence Listing.

3. ☐ The amendments have resulted in the cancellation of:

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

4. ☐ This report has been established as if (some of) the amendments annexed to this report and listed below had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).

☐ the description, pages _____

☐ the claims, Nos. _____

☐ the drawings, sheets/figs _____

☐ the sequence listing *(specify)*: _____

☐ any table(s) related to the sequence listing *(specify)*: _____

* *If item 4 applies, some or all of those sheets may be marked "superseded."*

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2003/001381

Box No. III Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been examined in respect of:

☐ the entire international application

☒ claims Nos. 16-21

because:

☒ the said international application, or the said claims Nos. 16-21 relate to the following subject matter which does not require an international preliminary examination *(specify)*:

See PCT Rule 67.1.(iv).: Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

☒ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. 16-19 are so unclear that no meaningful opinion could be formed *(specify)*:

Present claims 16-19 relate to use of an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and / or disclosure within the meaning of Article 5 PCT is to be found, however, for use of only a very small proportion of the compounds. In the present case, the claims so lack support, and the application so

.../...

☐ the claims, or said claims Nos. _____ are so inadequately supported by the description that no meaningful opinion could be formed.

☐ no international search report has been established for said claims Nos. _____

☐ the nucleotide and/or amino acid sequence listing does not comply with the standard provided for in Annex C of the Administrative Instructions in that:

the written form
- ☐ has not been furnished
- ☐ does not comply with the standard

the computer readable form
- ☐ has not been furnished
- ☐ does not comply with the standard

☐ the tables related to the nucleotide and/or amino acid sequence listing, if in computer readable form only, do not comply with the technical requirements provided for in the Annex C-*bis* of the Administrative Instructions.

☒ See Supplemental Box for further details.

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box No. III

lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible.

Consequently, the search of claims 16-19 has been carried out only for those parts which appear to be supported and disclosed, namely the parts relating to the compounds in present claims 20-21 and 24-26, as well as to the compounds numbered 38-56 in Fig. 10 (page 11/12) of the application.

INTERNATIONAL PRELIMINARY REPORT ON PATENTABILITY

International application No.
PCT/SE2003/001381

Box No. V **Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement**

1. Statement

Novelty (N)	Claims	1-15, 22-23	YES
	Claims	24-26	NO
Inventive step (IS)	Claims	-	YES
	Claims	1-15, 22-26	NO
Industrial applicability (IA)	Claims	1-15, 22-26	YES
	Claims	-	NO

2. Citations and explanations (Rule 70.7)

Reference is made to the following documents:

D1) US 6136542 A.
D2) Forsberg A, Rosqvist R. *In Vivo* Expression of Virulence Genes of *Yersinia pseudotuberculosis*. Infect Agents Dis 1993 Aug;2(4):275-278.
D3) Motin V L. *et al*. Genome-Wide Expression Profiling of *Yersinia pestis* During Low-Calcium Response. 102nd General Meeting of the American Society for Microbiology; Salt Lake City, UT, USA; May 19-23, 2002.
D4) Bolin I, Wolf-Watz H. The plasmid-encoded Yop2b protein of *Yersinia pseudotuberculosis* is a virulence determinant regulated by calcium and temperature at the level of transcription. Mol Microbiol 1988 Mar;2(2):237-245.
D5) Bolin I, Wolf-Watz H. Molecular cloning of the temperature-inducible outer membrane protein 1 of *Yersinia pseudotuberculosis*. Infect Immun 1984 Jan;43(1):72-78.
D6) Database CAPLUS; AN 1968:95469.
D7) Database CAPLUS; AN 1978:94851.
D8) Database CAPLUS; AN 1979:16910.
D9) Database CAPLUS; AN 1992:645585.
D10) GB 2365426 A.
D11) WO 02/43668 A2.
D12) Database CAPLUS; AN 1977:189559.
D13) Database CAPLUS; AN 1985:487603.
D14) Database CAPLUS; AN 2002:298309.
D15) Macielag M J. *et al*. Substituted Salicylanilides as Inhibitors of Two-Component Regulatory Systems in Bacteria. J Med Chem 1998, 41, 2939-2945.
D16) Database CAPLUS; AN 2000:780229.
D17) Database CAPLUS; AN 2002:11847.
D18) Database CAPLUS; AN 1990:440580.
D19) Database CAPLUS; AN 1968:458923.
D20) Database CAPLUS; AN 2000:72793.
D21) Database CAPLUS; AN 1999:184132.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Box No. V

D22) Database REGISTRY; CAS RN:s 400054-03-7, 305338-58-3, 303084-13-1, 416883-56-2 and 341979-94-0.
D23) Database CAPLUS; AN 1998:147346.
D24) Database CAPLUS; AN 1971:74914.

The present application relates to a method and a probe for identifying bacterial virulence modifying agents that affect the type III secretion machinery, agents thus identified, and their use. In the method of screening a *Yersinia* low-calcium response (LCR) system, with a luxAB construct as reporter gene, is used.

D1, which is considered to be the most relevant prior art regarding present claims 1-15 and 22-23, discloses a method for screening agents that activate or inhibit type III secretion machinery. The method comprises exposing gram-negative bacterial cells to a sample of an agent to be screened, which cells contain a reporter gene, such as the luxAB gene, transcriptionally fused to a promoter of a gene regulated by the type III secretion machinery, and detecting the presence or activity of the product of the reporter gene.

The detection indicates whether the sample activates or inhibits type III secretion machinery. The gram-negative bacteria are selected from, i.a., *Yersinia* (see specially the claims).

The technical difference in terms of claimed technical features, according to claims 1-15 and 22-23, between the present application and D1 is that the screening method of claimed invention is specifically adapted to a system where bacteria with a LCR are used (i.a. *Yersinia*).

The problem that this technical difference solves is, therefore, to adopt the screening method of D1 to a LCR system.

A solution to the problem of adopting the screening method of D1 to a LCR system can be found in D2 and D3. D2 and D3 disclose *in vitro* virulence plasmid expression systems, with maximal expression and secretion at 37 °C in medium lacking calcium (LCR systems). Since no surprising technical effect has been shown it is considered obvious for a person skilled in the art, with the guidance of D2 and D3, to adopt the method of D1 to a LCR system.

Accordingly, the invention according to claims 1-15 and 22-23 is not considered to involve an inventive step.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Previous Supplemental Box.

D6 and D16-D22 disclose compounds that are included by present claims 24-26 (e.g. Compounds 20, 22-25, 31, 35-37, 62 and others). The compounds of D6 and D16-D19 are reported with antibacterial activity.

The invention according to present claims 24-26 thus lacks novelty in view of each of the documents D6 and D16-D22.

Note that it is of no concern here that the claims mention that the compounds should be "capable of reducing virulence in Gram-negative bacteria having a type III secretion (TTS) system", since compound claims are directed to the compounds *per se* and cannot be restricted by mentioning desired properties of the compounds.

D8, D10-D15 and D23 disclose antibacterial compounds that are structurally very similar to the compounds of present claims 24-26. For example, there are compounds of claims 24-26 that differ from the compounds of D8, D10-D15 and D23 only in terms of one or two simple substituents on ring A. In view of each of said documents, the compounds of claims 24-26 are therefore considered to represent obvious alternatives to the person skilled in the art, looking for a solution to the general problem of finding alternative antibacterial compounds.

Since the compounds of the application are purported to decrease virulence in Gram-negative bacteria in particular, and since none of the prior art documents explicitly discloses such activity, but only antibacterial effect in general and/or activity against Gram-positive bacteria, it *may* be the case that a reformulation of the present claims 24-26 as a "second medical indication" (i.e., "Use of compounds... ...for the preparation of a medicament for use in the treatment of...") could be shown to be inventive. However, this would require convincing support for the notion that the activity against Gram-negative bacteria is an unexpected property of the known antibacterial compounds to the person skilled in the art.

As is implied from the arguments above, the invention according to present claims 24-26 is considered to lack an inventive step in view of each of the documents D6, D8, D10-D19 and D23.

The present claim 25 is an identical copy of claim 24. It is recognised here that the compounds probably intended to be disclosed in claim 25 are the compounds 38-56 in Fig. 10 (page 11/12) of the application. Of these compounds, Compound 42 is disclosed in D8, Compound 50 is disclosed in D17, Compound 51 is disclosed in D6, Compounds 38, 41 and 43 are disclosed in D22, Compound 55 is disclosed in D23, and Compound 56 is disclosed in D24.

.../...

Supplemental Box

In case the space in any of the preceding boxes is not sufficient.
Continuation of: Previous Supplemental Box.

The documents D4-D5, D7 and D9, cited in the International Search Report, are no longer considered to be of any particular relevance due to amendments of the claims.

In summary, the invention according to present claims 24-26 lacks novelty, and the invention according to present claims 1-15 and 22-26 is considered to lack an inventive step.

Box No. VIII Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

The present claim 25 is an identical copy of claim 24. It is recognised that the compounds probably intended to be disclosed in claim 25 are the compounds 38-56 in Fig. 10 (page 11/12) of the application.

CLAIMS

1. A method for identifying antibacterial agents comprising: depleting bacteria of a strain comprising a luxAB construct of Ca^{2+}, incubating the Ca^{2+} depleted bacteria with an agent the antibacterial effect of which shall be determined, recording the light emitted by the bacteria upon addition of an aldehyde, the incubation being carried out at a temperature which is at least 10° C higher than the temperature at which the light is emitted by the bacteria.

2. The method of claim 1, wherein the incubation temperature is at least 15° C higher than the temperature at which the light is emitted by the bacteria.

3. The method of claim 1 or 2, wherein said strain is a natural or mutant *Yersinia* sp. strain.

4. The method of claim 3, wherein the strain is a *Yersinia pseudotuberculosis* strain.

5. The method of claim 1, wherein the incubation temperature is about 37° C and the emission temperature is about 21° C, respectively.

6. A method for identifying antibacterial agents comprising:

- providing a *Yersinia* sp. bacterial strain comprising a luxAB construct;
- propagating the strain at room temperature in a Ca^{2+} depleting medium to obtain a suspension of Ca^{2+} depleted bacteria containing the luxAB construct;
- dissolving a measured amount of a sample of an antibacterial agent candidate in water, a mixture of water and of an organic solvent or an organic solvent;
organic solvent to prepare a solution of the agent;
- combining the solution of the agent with an aliquot of the bacterial suspension to obtain a test suspension;
- incubating the test suspension at a first temperature for a selected period of time;
- raising the temperature of the test suspension to a second temperature;
- continuing incubation at the second temperature for a selected period of time;
- lowering the temperature of the test suspension to a third temperature;

- continuing the incubation at the third temperature for a selected period of time;
- adding n-decanal or a functionally equivalent aldehyde to the test suspension;
- measuring light emitted from the test suspension over a period of time at the third temperature;
- quantifying the light emitted;
- calculating an antibacterial activity based on the quantity of emitted light;

wherein the first and third temperature is from 20° C to 26° C and the second temperature is about 37° C.

7. The method of claim 6, wherein the aldehyde is decanal or a functionally equivalent aldehyde.

8. The method of claim 6, wherein the aldehyde is added to the test suspension in form of an aqueous emulsion.

9. The method of claim 6, where in the measured amount of sample is selected to provide a concentration of the agent in the test suspension from 10 µg per mL to 100 µg per mL.

10. The method of claim 6, wherein the light emitted is less than 20 % of that emitted in an experiment in which no anti-bacterial agent had been added.

11. The method of claim 6, wherein the light emitted is less than 40 % of that emitted in an experiment in which no anti-bacterial agent had been added.

12. The method of claim 6, wherein the light emitted is less than 60 % of that emitted in an experiment in which no anti-bacterial agent had been added.

13. Use of a probe for identifying antibacterial agents comprising the *Yersinia pseudotuberculosis* strain pIB29EL in the method of claim 1-5 or claim 6-12.

14. Use of a probe for identifying antibacterial agents comprising a *Yersinia pseudotuberculosis* strain selected from pIB29AL, pIB102AL, optionally also from pIB102EL, in the method of claim 1-5 or claim 6-12.

15. Use of a probe for identifying antibacterial agents comprising a *Yersinia pseudotuberculosis* strain selected from pIB102FL and pIB102FΔhlhL in the method of claim 1-5 or claim 6-12.

16. Use of an agent comprising the structural element X–CO-NH-Y-Z, wherein X is aromatic or heteroaromatic carbon, Y is zero or -N=CH, and Z is unsubstituted or substituted aryl including heteroaryl for decreasing virulence in Gram-negative bacteria having a type III secretion (TTS) system.

17. Use of an agent for decreasing virulence in Gram-negative bacteria having a type III secretion (TTS) system of the general formula I

A- CO-NH-B (I)

wherein

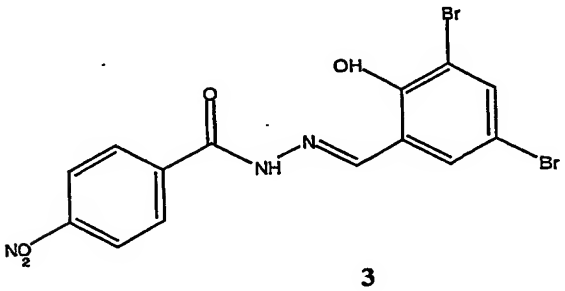
A is substituted or unsubstituted aryl or heteroaryl;

B is -X-Y, wherein X is zero or -N=CH- and Y is selected from unsubstituted aryl, unsubstituted heteroaryl, mono-, di- and tri-substituted aryl, mono-, di- and tri-substituted heteroaryl, with the proviso that, if X is -N=C-H-, Y is 2-hydroxyaryl.

18. The use of claim 17, wherein, if A is substituted aryl or heteroaryl, it is preferred to be mono- or disubstituted by one or more of halogen, nitro, hydroxy, alkoxy, C_1-C_6 alkyl, C_1-C_6 alkenyl.

19. The use of claim 17, wherein Y is selected from aryl and heteroaryl substituted with one or several of halogen, C_1-C_6 alkyl, C_1-C_6 alkenyl.

20. The use of a compound selected from:



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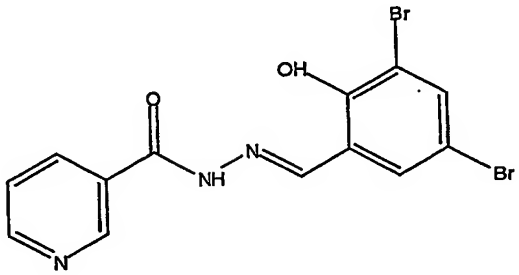

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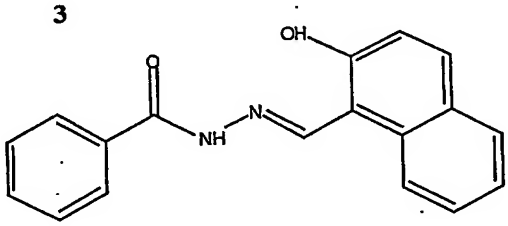

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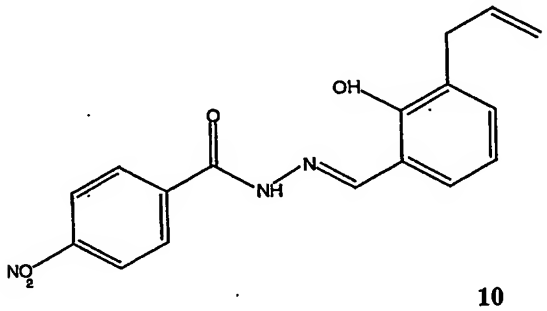

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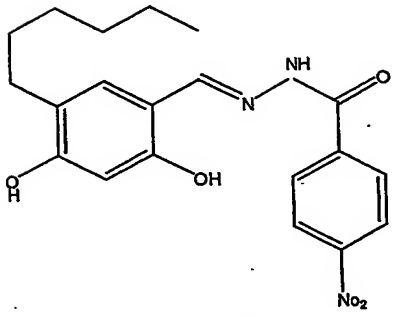

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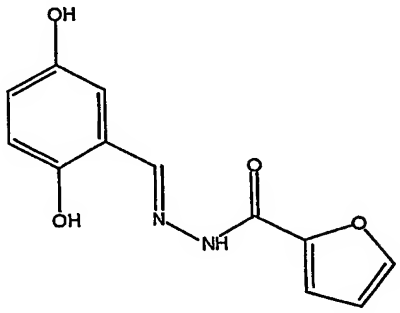

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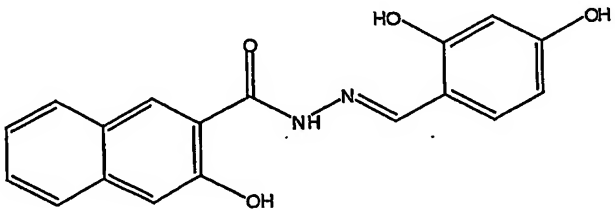
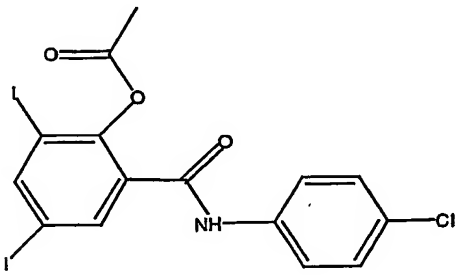


13

for reducing virulence in Gram-negative bacteria having a type III secretion (TTS) system.

AMENDED SHEET

21. The use of a compound selected from:



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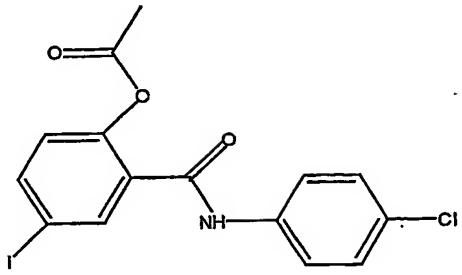

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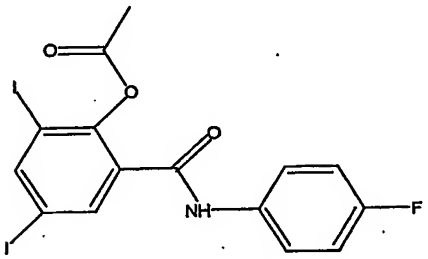

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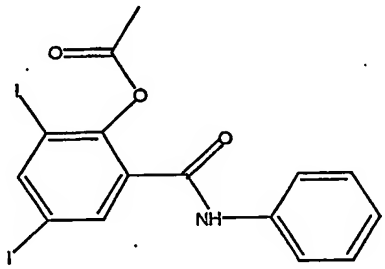

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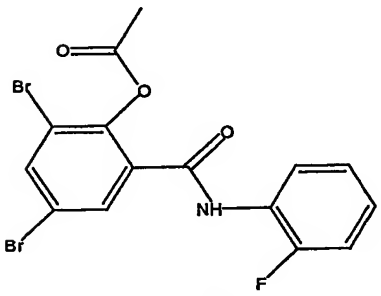

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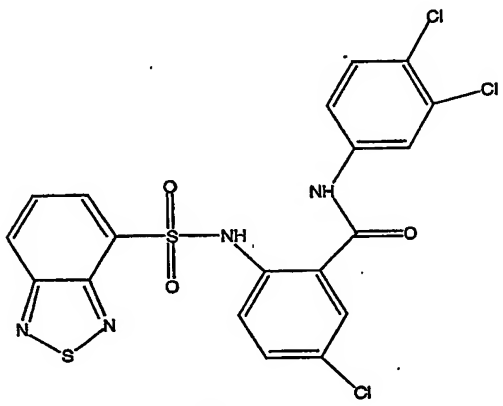


5

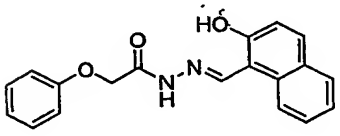
for reducing virulence in Gram-negative bacteria having a type III secretion (TTS) system

22. A method of screening for agents inhibiting virulence in Gram-negative bacteria having a type III secretion (TTS) system, the method being carried out in absence of eukaryotic cells, comprising contacting a gram-negative bacterium culture depleted in Ca^{2+}, in particular a *Yersinia* species, comprising a luxAB reporter gene construct, with a potentially bacterial virulence inhibiting agent, thereby forming a test suspension, manipulating the temperature of the test suspension and adding an aliphatic aldehyde to make the bacterium emit light, measuring the emitted light, comparing the amount of emitted light with the light emitted in absence of the bacterial virulence inhibiting or activating agent.

AMENDED SHEET

23. A method of screening for agents inhibiting virulence in Gram-negative bacteria having a type III secretion (TTS) system, the method being carried out in absence of eukaryotic cells, comprising contacting a gram-negative bacterium culture enriched in Ca^{2+}, in particular a *Yersinia* species, comprising a luxAB reporter gene construct, with a potentially bacterial virulence activating agent, thereby forming a test suspension, manipulating the temperature of the test suspension and adding an aliphatic aldehyde to make the bacterium emit light, measuring the emitted light, comparing the amount of emitted light with the light emitted in absence of the bacterial virulence inhibiting or activating agent.

24. A compound capable of reducing virulence in Gram-negative bacteria having a type III secretion (TTS) system selected from: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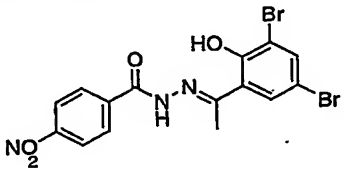
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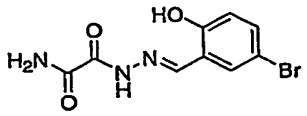
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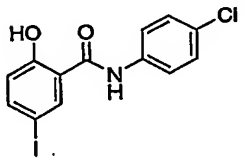
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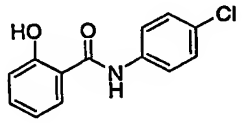
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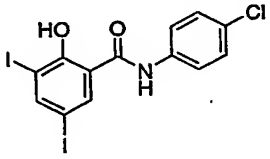
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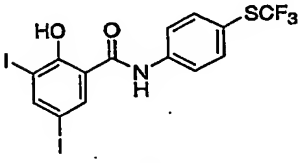
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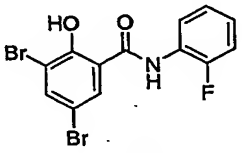
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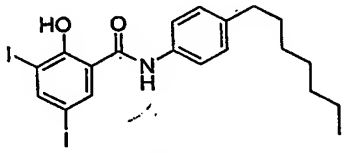
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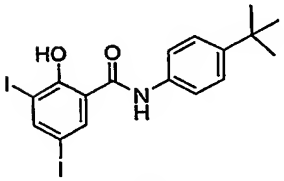
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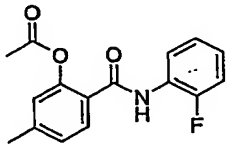
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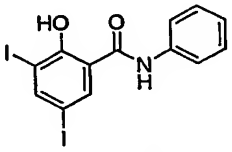
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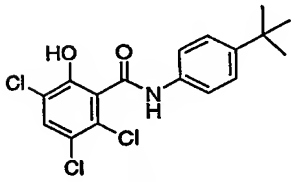
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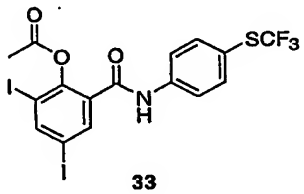
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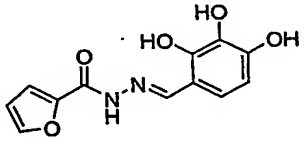
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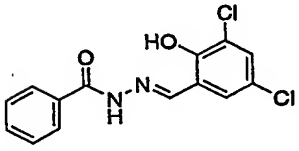
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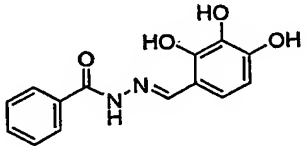
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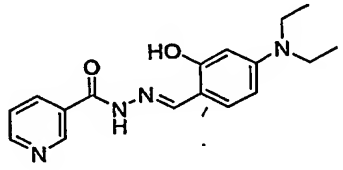
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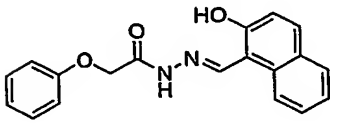
25. A compound capable of reducing virulence in Gram-negative bacteria having a type III secretion (TTS) system selected from:



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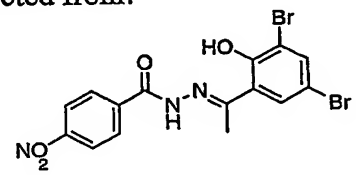

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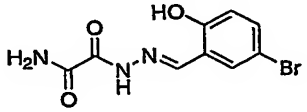

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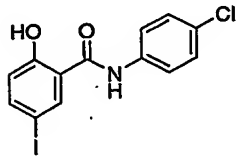

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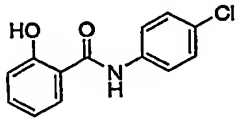

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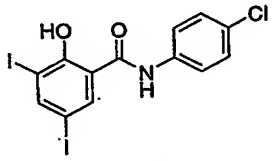

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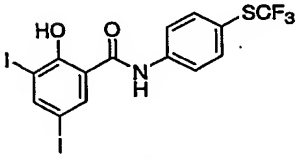

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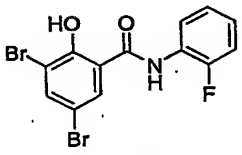

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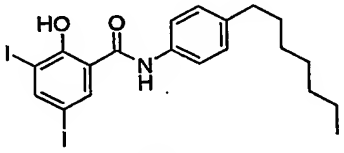

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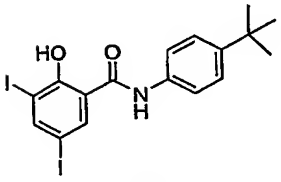

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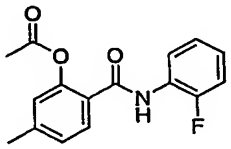

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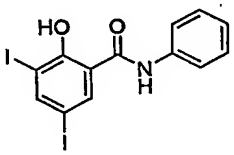

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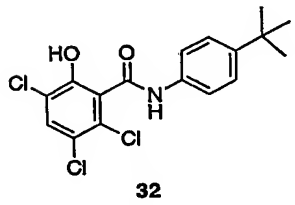

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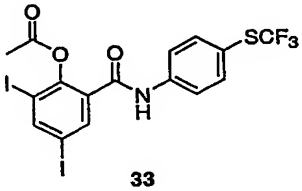

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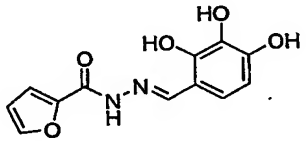

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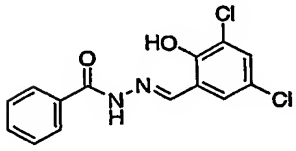

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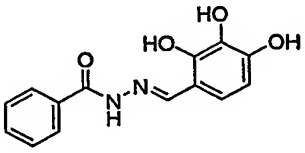

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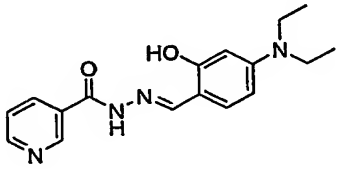

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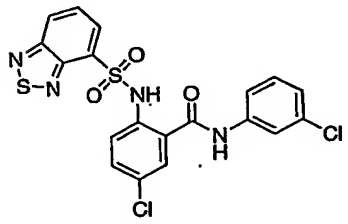
26. A compound capable of reducing virulence in Gram-negative bacteria having a type III secretion (TTS) system selected from:



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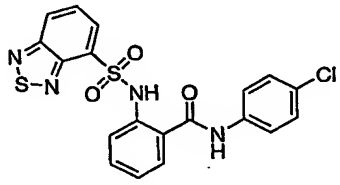

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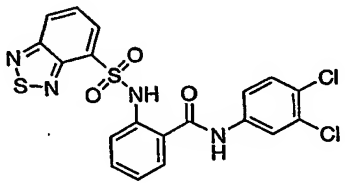

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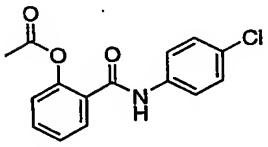

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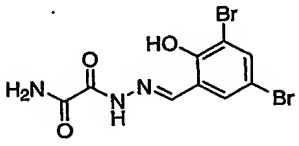

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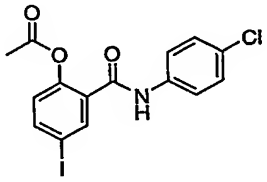

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